SOME PHENOLIC COMPOUNDS FROM LICHEN *PARMOTREMA SANCTI-ANGELII* (LYNGE) HALE (PARMELIACEAE)

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ABSTRACT

*Four known phenolic compounds 5,7-dihydroxy-1(3H)-isobenzofuranone **(1)**, methyl lecanorate **(2)**, orcinol **(3)**, and skyrin **(4)** were isolated from the lichen Parmotrema sancti-angelii* (Lynge) Hale*. Their chemical structures were established by 1D NMR, high resolution ESI–MS spectroscopic analysis and comparison with those reported in the literatures. This is the first time these compounds are reported in Parmotrema sancti-angelii* (Lynge) Hale.

Keywords: Parmotrema sancti-angelii, depside, monocyclic compounds, skyrin

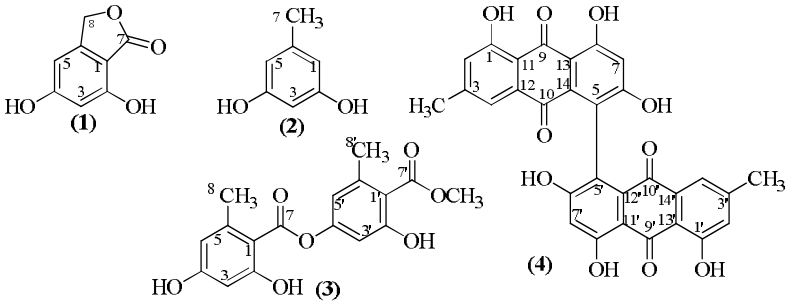

Figure 1. *Chemical structures of **1-4**.*

TÓM TẮT

Một số hợp chất phenol từ loài địa y Parmotrema sancti-angelii (Lynge) Hale *(Parmeliaceae)*

*Bốn hợp chất phenol 5,7-dihydroxy-1(3H)-isobenzofuranone **(1)**, methyl lecanorate **(2)**, orcinol **(3)** và skyrin **(4)** được cô lập từ loài địa y Parmotrema sancti-angelii (Lynge) Hale. Cấu trúc hóa học của chúng được xác định bằng các phương pháp phổ nghiệm cũng như so sánh với các tài liệu tham khảo. Đây là lần đầu tiên các hợp chất này được tìm thấy trong loài địa y Parmotrema sancti-angelii (Lynge) Hale.*

Từ khóa: Parmotrema sancti-angelii, depside, monocyclic compounds, skyrin.

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1. Introduction

Phenolic compounds from lichen are bioactive compounds with various activities according to Boustie & Grube (2007),[1] Boustie *et al.* (2010),[2] Muller (2001).[7] Seven phenolic compounds alectoronic acid, atranorin, α-collactolic acid, furmaprotocetraric acid, hypoprotocetraric acid, protocetraric acid, and lecanoric acid, demonstrating bactericidal activity from *Parmotrema sancti-angelii* were reported by Verma *et al*. (2011).[8] They possessed the common skeletons as depsidone, depside, and diphenyl ether.

Figure 2. *Parmotrema sancti-angelii (Lynge) Hale*

In this paper, from the lichen *Parmotrema sancti-angelii* collected in Lam Dong province, four known phenolic compounds 5,7-dihydroxy-1(3*H*)-isobenzofuranone **(1)**,[10] methyl lecanorate **(2)**,[8] orcinol **(3)**,[6] and skyrin **(4)**[3,5] were isolated by using efficient separation techniques. Their chemical structures were elucidated by spectroscopic data analysis and comparison with those reported in the literature.

2. Experimental

General experimental procedures

The NMR spectra were measured on a Bruker Avance III (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometers with TMS as internal standard. Proton chemical shifts were referenced to the solvent residual signal of $CDCl_3$ at δ_H 7.26, of CD_3COCD_3 at δ_H 2.05, of CD_3OD at δ_H 3.31. The ^{13}C–NMR spectra were referenced to the central peak of $CDCl_3$ at δ_C 77.1, of CD_3COCD_3 at δ_C 29.4, of CD_3OD at δ_C 49.0. The HR–ESI–MS were recorded on a Bruker micrOTOF Q-II. TLC was carried out on precoated silica gel 60 F_{254} or silica gel 60 RP–18 $F_{254}S$ (Merck) and spots were visualized by spraying with 30% H_2SO_4 solution followed by heating. Gravity column chromatography was performed with Silica gel 60 (0.040–0.063 mm, Himedia).

Plant material

Parmotrema sancti-angelii (Lynge) Hale was collected on the bark of tea trees *Camellia sinensis* at Bao Loc city, Lam Dong province, Vietnam (07/2013–09/2013) and the scientific name was identified by Dr. Harrie J. M. Sipman, Botanic Garden and Botany Museum Berlin-Dahlem, Freie University, Berlin, Germany. A voucher

specimen (No US-B021) was deposited in the herbarium of the Department of Organic Chemistry, Univeristy of Science, Vietnam National University - Ho Chi Minh City, Vietnam.

Extraction and isolation

The clean, air-dried and ground material (950 g) was extracted by maceration with acetone at ambient temperature, and the filtrated solution was evaporated under reduced pressure to afford the crude acetone extract (145.1 g). The crude acetone extract (145.1 g) was dissolved in hot acetone (45 ^{o}C) to obtain two parts, the solution and the insoluble powder (**P**, 30.0 g). The solution was evaporated to afford the acetone extract (110.4 g). This one was applied on normal phase silica gel column chromatography, eluted with the solvent system of hexane–ethyl acetate (9:1) to afford **H0** extract (6.1 g). Continuous elution of the column with the same solvent systems but increasing polarity (8:2), (7:3), (6:4), (4:6), and (3:7) yielded five fractions, **H1** (2.1 g), **C** (15.4 g), **EA1** (4.5 g), **EA2** (5.1 g), and **EA3** (9.8 g), respectively.

Extract **EA1** (4.5 g) was applied to silica gel column chromatography, eluted with hexane–ethyl acetate–acetone (9:1:0.5) to give four fractions, **EA1.1** (1.9 g) and **EA1.2**–**1.4** (2.1 g). Fraction **EA1.1** (1.9 g) was fractionated by column chromatography, eluting with hexane–ethyl acetate–acetic acid (9:1:0.5) to give two fractions, **EA1.1.1** (998.9 g) and **EA1.1.2** (219.5 mg). Fraction **EA1.1.1** was further chromatographed, eluted with hexane–ethyl acetate–acetone (9:1:0.5) to afford **1** (395.7 mg) and **2** (4.9 mg). Fraction **EA1.1.2** (219.5 mg) was purified, eluting with hexane–ethyl acetate–methanol (7:3:0.02) to obtain **3** (14.7 mg) and **4** (3.8 mg).

- **5,7-Dihydroxy-1(3*H*)-isobenzofuranone (1):** White amorphous powder. The ^{1}H-NMR data (Acetone-d_6): 6.38 (1H, brs, H-3), 6.53 (1H, brs, H-5), 5.22 (2H, s, H-8). The ^{13}C-NMR data (Acetone-d_6): 104.4 (C-1), 166.2 (C-2), 102.0 (C-3), 158.8 (C-4), 103.1 (C-5), 151.5 (C-6), 171.5 (C-7), 70.3 (C-8). These spectroscopic data were suitable with those reported in the literature.[10]

- **Methyl lecanorate (2)**: White amorphous powder. The ^{1}H-NMR data ($CDCl_3$): 6.30 (1H, d, J=2.0 Hz, H-3), 6.32 (1H, d, J=2.0 Hz, H-5), 2.61 (3H, s, H-8), 6.59 (1H, d, J=2.0 Hz, H-3'), 6.71 (1H, d, J=2.0 Hz, H-5'), 2.57 (3H, s, H-8'), 3.98 (-COO**C**H$_3$), 11.56 (s, 2-OH), 11.28 (s, 2'-OH). The ^{13}C-NMR data ($CDCl_3$): 110.5 (C-1), 166.6 (C-2), 101.4 (C-3), 164.2 (C-4), 108.9 (C-5), 144.2 (C-6), 172.2 (C-7), 24.6 (C-8), 101.3 (C-1'), 161.0 (C-2'), 112.3 (C-3'), 154.2 (C-4'), 116.5 (C-5'), 143.6 (C-6'), 169.5 (C-7'), 24.9 (C-8'), 52.4 (-OCH$_3$). These spectroscopic data were suitable with with those reported in the literatures.[8]

- **Orcinol (3):** White amorphous powder. The ^{1}H-NMR data ($CDCl_3$): 6.23 (2H, d, J=1.5 Hz, H-1 & H-5), 6.16 (1H, brs, H-3), 2.24 (3H, s, H-7). These spectroscopic data were suitable with with those reported in the literature.[6]

- **Skyrin (4):** Red amorphous powder. HR-ESI-MS *m/z* 537.0813 [M-H]- (calcd. for $C_{30}H_{17}O_{10}$, 537.0822). The ^{1}H-NMR data ($CDCl_3$): 7.05 (2H, d, J=2.0 Hz, H-2 & H-2'), 7.35 (2H, J=1.5 Hz, H-4 & H-4'), 6.90 (2H,s, H-7 & H-7'), 2.34 (6H, s, 3-CH_3 & 3'-CH_3), 11.96 (s, 8-OH & 8'-OH), 12.84 (s, 1-OH & 1'-OH). The ^{13}C-NMR data (Acetone-d_6): 163.0 (C-1 & C-1'), 124.4 (C-2 & C-2'), 149.2 (C-3 & C-3'), 121.1 (C-4 & C-4'), 123.6 (C-5 & C-5'), 165.2 (C-6 & C-6'), 108.4 (C-7 & C-7'), 166.1 (C-8 & C-8'), 191.5 (C-9 & C-9'), 183.2 (C-10 & C-10'), 132.8 (C-11 & C-11'), 110.9 (C-12 & C-12'), 114.0 (C-13 & C-13'), 134.7 (C-14 & C-14'), 21.9 (3-CH_3 & 3'-CH_3). These spectroscopic data were suitable with with those reported in the literature.[3,5]

3. Results and discussion

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum exhibited signals for one oxymethylene group at 5.22 (2H, *s*, H_2-8), two aromatic methine protons at δ_H 6.38 (1H, *brs*) and 6.53 (1H, *brs*). The chemical shift of H_2-8 shifted to low field indicated that this methylene group linked to a phenyl group and an ester group. The structure of **1** was confirmed by the ^{13}C NMR data. The spectral data were suitable to the published ones,[10] therefore, **1** was 5,7-dihydroxy-1(3*H*)-isobenzofuranone.

Compound **2** was obtained as a white amorphous powder. The ^{1}H-NMR showed two singlets at δ_H 2.57 (3H) and 2.61 (3H) for methyl groups, a pair of doublets for two aromatic protons at δ_H 6.30 and 6.32 (each 1H, J=2.0Hz) for proton H-5 and H-3 and a pair of doublets at δ_H 6.59 and 6.71 (each 1H, J=2.0Hz) for proton H-5' and H-3', respectively. The ^{13}C-NMR showed 16 carbon signals including two carboxyl groups (-COO), four methine carbons, two methyls and eight aromatic substituted carbons. Comparison of the spectroscopic NMR data of **2** with those of methyl lecanorate[8] suggested that they were similar. Accordingly, **2** was elucidated as methyl lecanorate.

Compound **3** was obtained as a white amorphous powder. The ^{1}H-NMR ($CDCl_3$) spectrum displayed signals of one methyl group at δ 2.24 (3H, *s*), three methine protons at δ 6.23 (2H, *d*, *J* = 1.7 Hz, H-4 and H-6), 6.16 (1H, *t*, *J* = 1.7 Hz, H-2). Analysis of the ^{1}H-^{1}H spin-spin interaction of three aromatic protons revealed that **3** contained 1,3,5-trisubstituted benzene ring. These spectroscopic data were suitable to the published data, [6] therefore **3** was determined as orcinol.

Compound **4** was obtained as red amorphous powder. The ^{1}H-NMR spectrum showed one methyl group δ_H 2.34, a pair of doublets for two aromatic protons at δ_H 7.05 and 7.35 (each 1H, J=2.0Hz), a singlet aromatic proton at δ_H 6.90, and two chelated hydroxyl groups at δ_H 11.96 and 12.84. These findings suggested that **4** possessed an anthraquinone skeleton. The ^{13}C-NMR spectrum showed the signals of 14 carbons corresponding to one methyl, three aromatic methines, and ten quaternary carbons including three oxygenated and two carbonyl carbons. However, the HR-ESIMS established the molecular formula of **4** to be $C_{30}H_{18}O_{10}$, indicating a symmetrical structure. Accordingly, **4** was established as a bianthraquinone skyrin.[3,5]

4. Conclusion

Four known phenolic compounds were also isolated from the lichen *Parmotrema sancti-angelii* collected in Lam Dong province. This is the first time these compounds are reported in *Parmotrema sancti-angelii (Lynge) Hale.* Further studies on this lichen are in progress.

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